CLAIMS

1. A TY145 like subtilase which is at least 63% homologous to the sequence of SEQ ID NO: 1, comprising the overall subtilisin fold and the following structural characteristics:

a) a twisted beta-sheet with 7 strands,

b) six alpha helices,

c) at least three ion-binding sites,

wherein the Strong ion-binding site of the BPN' like subtilases is not present, and with the exception of the TY145 subtilase, the S39 subtilase from TA39, the S41 subtilase from TA41, and sphericase from *B. sphaericus*.

2. The subtilase of claim 1, wherein the positions of said three ion-binding sites in the three-dimensional structure of the subtilase is defined by the distance to the c-alpha atoms of the three active site amino acid residues of the subtilases, that is Ser, His and Asp, and the c-alpha atom of the amino acid residue next to the active site Ser residue (next to Ser), wherein said distances between:

a) the Weak ion-binding site and i) Asp c-alpha atom is 17.50-19.50 Å, ii) His c-alpha atom is 21-23 Å, iii) Ser c-alpha atom is 13.80-15.80 Å, iv) next to Ser c-alpha atom is 15.80-17.80 Å,

b) the Far ion-binding site and i) Asp c-alpha atom is 28.70-30.70 Å, ii) His c-alpha atom is 28-30 Å, iii) Ser c-alpha atom is 20-22 Å, iv) next to Ser c-alpha atom is 19.50-21.50 Å,

c) the Near ion-binding site and i) Asp c-alpha atom is 27-29 Å, ii) His c-alpha atom is 29.50-31.50 Å, iii) Ser c-alpha atom is 21.40-23.40 Å, iv) next to Ser c-alpha atom is 22.50-24.50 Å.

3. A subtilase of claim 2, wherein the positions of the three ion-binding sites are defined by the distance to the c-alpha atoms of amino acid residues D35, H72, S251 and M252 of SEQ ID NO: 1 or by the distances to the c-alpha atoms of equivalent amino acid residues in another subtilase of the invention in accordance with claim 1, wherein the distance between

a) the Weak ion-binding site and i) D35 c-alpha atom is 18.55 Å, ii) H72 c-alpha atom is 21.98 Å, iii) S251 c-alpha atom is 14.71 Å, iv) M252 c-alpha atom is 16.75 Å,

b) the Far ion-binding site and i) D35 c-alpha atom is 29.68 Å, ii) H72 c-alpha atom is 29.10 Å, iii) S251 c-alpha atom is 20.96 Å, iv) M252 c-alpha atom is 20.35 Å,

c) the Near ion-binding site and i) D35 c-alpha atom is 28.04 Å, ii) H72 c-alpha atom is 30.43 Å, iii) S251 c-alpha atom is 22.28 Å, iv) M252 c-alpha atom is 23.58 Å,
and wherein the variation on the above mentioned distances are ±0.8 Å, preferably ±0.7 Å, more preferably ±0.6 Å, more preferably ±0.5 Å, more preferably ±0.4 Å, or most preferably ±0.3 Å.

4. A method of producing a variant of a parent TY145 like subtilase, the variant having at least one altered property as compared to the parent TY145 like subtilase, the method comprising:

a) modelling the parent TY145 like subtilase on the three-dimensional structure of a TY145 subtilase to produce a three-dimensional structure of the parent TY145 like subtilase;

b) comparing the three-dimensional structure obtained in step a) to the three-dimensional structure of a TY145 subtilase;

c) identifying on the basis of the comparison in step b) at least one structural part of the parent TY145 subtilase, wherein an alteration in said structural part is predicted to result in an altered property;

d) modifying the nucleic acid sequence encoding the parent TY145 subtilase to produce a nucleic acid sequence encoding deletion or substitution of one or more amino acids at a position corresponding to said structural part, or an insertion of one or more amino acid residues in positions corresponding to said structural part; and

e) expressing the modified nucleic acid sequence in a host cell to produce the variant TY145 subtilase.

5. A method of claim 4, wherein the TY145 subtilase on which the parent TY145 subtilase is modelled in step a) is at least 63% homologous to SEQ ID NO: 1, preferably at least 65% homologous, more preferably at least 70%, more preferably at least 74%, more preferably at least 80%, more preferably at least 83%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% or even more preferably at least 99% homologous to the sequence of SEQ ID NO: 1.

6. A method of claim 4 or 5, wherein the TY145 subtilase on which the parent TY145 subtilase is modelled in step a) is defined in accordance with claim 3.

7. A method of producing a variant of a parent Subtilisin family subtilase, the variant having at least one altered property as compared to the parent Subtilisin family subtilase, the method comprising:

a) modelling the parent Subtilisin family subtilase on the three-dimensional structure of a Subtilisin family subtilase to produce a three-dimensional structure of the parent Subtilisin family subtilase;

b) comparing the three-dimensional structure obtained in step a) to the three-dimensional structure of a TY145 like subtilase;

c) identifying on the basis of the comparison in step b) at least one structural part of the parent Subtilisin family subtilase, wherein an alteration in said structural part is predicted to result in an altered property;

d) modifying the nucleic acid sequence encoding the parent Subtilisin family subtilase to produce a nucleic acid sequence encoding deletion or substitution of one or more amino acids at a position corresponding to said structural part, or an insertion of one or more amino acid residues in positions corresponding to said structural part; and

e) expressing the modified nucleic acid sequence in a host cell to produce the variant Subtilisin family subtilase.

8. A method of claim 7, wherein the Subtilisin family subtilase on which the parent Subtilisin family subtilase is modelled in step a) is at least 61% homologous to SEQ ID NO: 5, preferably at least 63% homologous, preferably at least 65% homologous, more preferably at least 70%, more preferably at least 74%, more preferably at least 80%, more preferably at least 83%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% or even more preferably at least 99% homologous to the sequence of SEQ ID NO: 5.

9. A method of claim 7 or 8, wherein the TY145 subtilase of step b) is defined in accordance with claim 3.

10. A method of any of claims 7-9, wherein the TY145 subtilase in step b) is at least 63% homologous with the sequence of SEQ ID NO: 1, preferably at least 65% homologous, more preferably at least 70%, more preferably at least 74%, more preferably at least 80%, more

preferably at least 83%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% or even more preferably at least 99% homologous to the sequence of SEQ ID NO: 1.

11. A method of producing a variant of a parent TY145 like subtilase, the variant having at least one altered property as compared to the parent TY145 like subtilase, the method comprising:

a) modelling the parent TY145 like subtilase on the three-dimensional structure of a TY145 like subtilase to produce a three-dimensional structure of the parent TY145 like subtilase;

b) comparing the three-dimensional structure obtained in step a) to the three-dimensional structure of a Subtilisin family subtilase;

c) identifying on the basis of the comparison in step b) at least one structural part of the parent TY145 like subtilase, wherein an alteration in said structural part is predicted to result in an altered property;

d) modifying the nucleic acid sequence encoding the parent TY145 like subtilase to produce a nucleic acid sequence encoding deletion or substitution of one or more amino acids at a position corresponding to said structural part, or an insertion of one or more amino acid residues in positions corresponding to said structural part; and

e) expressing the modified nucleic acid sequence in a host cell to produce the variant TY145 like subtilase.

12. A method of claim 11, wherein the Subtilisin family subtilase of step b) is at least 61% homologous to SEQ ID NO: 5, preferably at least 63% homologous, preferably at least 65% homologous, more preferably at least 70%, more preferably at least 74%, more preferably at least 80%, more preferably at least 83%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% or even more preferably at least 99% homologous to the sequence of SEQ ID NO: 5.

13. A method of claim 11 or 12, wherein the parent TY145 like subtilase is defined in accordance with claim 3.

14. A method of any of claims 11-13, wherein the parent TY145 like subtilase is at least 63% homologous with the sequence of SEQ ID NO: 1, preferably at least 65% homologous, more preferably at least 70%, more preferably at least 74%, more preferably at least 80%, more preferably at least 83%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% or even more preferably at least 99% homologous to the sequence of SEQ ID NO: 1.

15. A variant subtilase comprising an alteration in one or more positions located at a distance of not more than 10 Å to one of the ion-binding sites of TY145, wherein the positions, as specified in SEQ ID NO: 1, located at a distance of not more than 10 Å to:

a) the Weak ion-binding site are: 154, 155, 158, 164, 165, 166, 167, 168, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 211, 220, 221, 222, 223, 224, 225, 226, 227, 228, 277, 281 and 305,

b) the Near ion-binding site are: 185, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 277, 281, 299, 300, 301, 304, 305,

c) the Far ion-binding site are: 193, 198, 199, 201, 202, 204, 216, 217, 219, 226, 227, 228, 229, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306 and 307.

16. A subtilase variant of claim 15, wherein the alterations are one or more of the substitutions I220S,T,D,E; T215S,D,E; G298A,S,T,D,E; G296A,S,T,D,E; V185T,D,E; I221N,D,T,E.

17. A TY145 like subtilase variant comprising the introduction of a ion-binding site corresponding to the Strong ion-binding site of the Subtilisin family subtilases, wherein said variant has a deletion of the region H83-G90, or at least one deletion of one amino acid residue in the region H83-G90, of SEQ ID NO: 1 and subsequent insertion of one or more amino acid residues, preferably insertion of the sequence LNNSIG (SEQ ID NO: 48) between residues A82 and V91.

18. A TY145 like subtilase variant in which one or more ion-binding sites have been removed, wherein said variant comprises one or both of the alterations

a) deletion of the region K290-D300, or at least one deletion of one amino acid residue in the region K290-D300 of SEQ ID NO: 1 and subsequent insertion of one or more amino acid residues, preferably insertion between I289 and Y301 of the sequence GDS (SEQ ID NO: 49) or DST (SEQ ID NO: 50), and preferably further comprising the substitution S303Y,

b) deletion of the region N212-R224, or at least one deletion of one amino acid residue in the region N212-R224 of SEQ ID NO: 1 and subsequent insertion of one or more amino acid residues, preferably insertion of a proline residue or an alanine residue between G211 and D225.

19. A TY145 like subtilase variant comprising one or more alterations in one or more of the positions contained in the following highly mobile regions:
84, 85, 86, 87 and 88,
108, 109, 110, 111, 112, 113, 114, 115, 116 and 117,
141, 142, 143, 144, 145 and 146,
150, 151 and 152,
169, 170 and 171,
200 and 201,
211, 212, 213, 214, 215, 216, 217, 218, 219 and 220,
242 and 243,
268, 269 and 270.

20. A TY145 like subtilase variant comprising one or more alterations in one or more of the positions contained in the following mobile regions:
1, 2, 3, 4, 5, 6 and 7,
17, 18, 19, 20, 21, 22 and 23,
38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50,
57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68 and 69,
84, 85, 86, 87, 88, 89, 90, 91 and 92,
107, 108, 109 and 110,
239, 240, 241, 242 and 243
265 and 266,

wherein said alterations preferably are introduced in one or both of the regions 57-69 and 84-92.

21. A TY145 like subtilase variant comprising one or more disulfide bridges introduced by one or more of the following modifications: G26C+A95C; A167C+T254C; R203C+G292C; V228C+A284C, wherein the positions corresponds to the positions in SEQ ID NO: 1.

22. A TY145 like subtilase variant comprising the substitution D116H,K,R.

23. A TY145 like subtilase variant comprising an alteration in one or more of the positions 18, 115, 185, 269 and 293 of SEQ ID NO: 1, wherein the preferred alterations are Q18P, D115P, V185P, T269P and I293P.

24. A TY145 like subtilase variant comprising an alteration in one or more of the positions contained in the following regions:
16, 17, 18, 19, 20, 21 and 22,
40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72 and 73,
118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130 and 131,
140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157,158, 159, 160 and 161,
275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293 and 294,
wherein such alterations preferably are made in one or both the regions 40-73 and 140-161, preferably in the sub-regions 65-73 and 140-150.

25. A TY145 like subtilase variant comprising an alteration in one or more of the following positions: 35, 36, 70, 72, 106, 109, 110, 111, 112, 113, 114, 117, 139, 140, 141, 142, 143, 144, 145, 147, 150, 167, 168, 169, 170, 171, 172, 173, 174, 177, 180, 207, 239, 247, 248, 249, 250, 251 and 252 of SEQ ID NO: 1.

26. A TY145 like subtilase variant comprising an alteration in one or more of the following positions: V31, V38, T79, V80, L81, V188, T254, preferably one or more of V31I, T79S and V80A.

27. A TY145 like subtilase variant comprising an alteration of an Asn-Gly sequence by deletion or substitution of at least one of the Asn or Gly residues, preferably the Asn residue.

28. The variant of claim 27 comprising substitution of the Asn and/or Gly residue with an amino acid residue selected from the group consisting of A, Q, S, P, T and Y.

29. The variant of claim 28, wherein the substitution is performed in one or more of the following positions:
B. sphaericus: 198-199, 240-241
TY145: 87-88, 109-110, 199-200
TA41: 83-84, 198-199
TA39: 88-89, 198-199.

30. A TY145 like subtilase variant comprising an alteration of a tyrosine residue by deletion or substitution, preferably to phenylalanine.

31. The variant of claim 30, wherein the substitution is performed in one or more of the following positions:
B. sphaericus: 14, 91, 102, 112, 155, 157, 172, 179, 201, 206, 211, 218, 235, 239, 243, 292, 300,
TY145: 15, 39, 92, 103, 113, 156, 158, 202, 219, 240, 244, 287, 301, 307,
TA41: 15, 91, 102, 112, 155, 157, 179, 201, 218, 235, 243,
TA39: 15, 61, 91, 102, 112, 155, 157, 173, 179, 201, 211, 218, 235, 243, 267, 281, 284, 292, 293, 296.

32. A TY145 like subtilase variant comprising an alteration of a methionine residue residue by deletion or substitution, preferably to a serine or alanine residue.

33. The variant of claim 32, wherein the substitution is performed in one or more of the following positions:
B. sphaericus: 138, 251,
TY145: 139, 252,
TA41: 1, 138,251,
TA39: 1, 138, 251.

34. A Subtilisin family subtilase variant in which the Strong ion-binding site has been removed, wherein said variant comprises deletion of the region L75-G80 (BPN' numbering), or at least one deletion of one amino acid residue in the region L75-G80 (BPN' numbering), or a corresponding region in another Subtilisin family subtilase, and subsequent insertion of one or more amino acid residues, preferably insertion of the sequence GGSNG (SEQ ID NO: 51) of positions 84-88 of TY145 (SEQ ID NO: 1) between A74 and V81, and preferably further comprising one or both of the substitutions L80Y and Q2A,N.

35. A BPN' like subtilase variant comprising one or more of the alterations V28I,A,L; I35V,A,L; T71S; I72A,G,V; A73L,G; M175V,A; and T224S,A, wherein preferred variants of Savinase comprises one or more of the substitutions V28I, I35V, T71S, I72A, A73L, M175V and T224S (BPN' numbering), especially variants comprising the combinations V28I+I35V, V28I+T71S, V28I+I72A, V28I+A73L, V28I+M175V, I35V+T71S, I35V+I72A, I35V+A73L, I35V+A73L, I35V+M175V, T71S +I72A, T71S +A73L, T71S +A73L, T71S +M175V, I72A +A73L, I72A +A73L, I72A +M175V, A73L +M175V.

36. A BPN' like subtilase variant comprising one or more of the following alterations:

a) deletion of residues PSPSATLEQAVN (SEQ ID NO: 23) (positions 129-140) in Savinase (BPN' numbering) and subsequent insertion of residues SAKDSLIASAVD (SEQ ID NO: 22) (positions 144-155) from TY145 between S128 and S141 in Savinase,

b) deletion of residues SGNSGAGSISYPARYA (SEQ ID NO: 25) (positions 153-172) in Savinase (BPN' numbering) and subsequent insertion of residues AGNSGSGSNTIGFPGGLV (SEQ ID NO: 24) (positions 168-185) from TY145 between A152 and N173 in Savinase,

c) deletion of residues VNVQSTYPGSTYASLN (SEQ ID NO: 27) (positions 203-218) in Savinase (BPN' numbering) and subsequent insertion of residues ASVESTWYTGGYNTIS (SEQ ID NO: 26) (positions 233-248) from TY145 between G202 and G219 in Savinase.

37. A BPN' like subtilase variant comprising one or more of the following alterations:

a) deletion of residues LSLGSPS (SEQ ID NO: 29) (positions 124-130) in Savinase (BPN' numbering) and subsequent insertion of residues MSLGSSG (SEQ ID NO: 28) (positions 138-144) from TA39 subtilase between N123 and P131 in Savinase,

b) deletion of residues LSLGSPSPSATL (SEQ ID NO: 31) (positions 124-135) in Savinase variant V104S (BPN' numbering) and subsequent insertion of residues MSLGSSGESSLI (SEQ ID NO: 30) (positions 138-149) from TA39 subtilase between N123 and E136 in Savinase variant V104S.

38. A BPN' like subtilase variant comprising one or more of the following alterations:

a) deletion of residues VQAPAAHN (SEQ ID NO: 33) (positions 11-18) in Savinase (BPN' numbering) and subsequent insertion of residues NNSSITQT (SEQ ID NO: 32) (positions 16-23) from TA39 subtilase between R10 and R19 in Savinase,

b) deletion of residues VPG*EPST (SEQ ID NO: 35) (positions 51-58) in Savinase (BPN' numbering) and subsequent insertion of residues TVGTTYTN (SEQ ID NO: 34) (positions 56-63) from TA39 subtilase between F50 and Q59 in Savinase,

c) deletion of residues GN (positions 61-62) in Savinase (BPN' numbering) and subsequent insertion of residues RQ (positions 69-70) from TA39 subtilase between D60 and G63 in Savinase.

d) deletion of residues PSPSATL (SEQ ID NO: 37) (positions 129-135) in Savinase (BPN' numbering) and subsequent insertion of residues SGESSLI (SEQ ID NO: 36) (positions 143-149) from TA39 subtilase between S128 and E136 in Savinase.

e) deletion of residues YPGSTYASL (SEQ ID NO: 39) (positions 209-217) in Savinase (BPN' numbering) and subsequent insertion of residues WFDGGYATI (SEQ ID NO: 38) (positions 238-246) from TA39 subtilase between T208 and N218 in Savinase.

39. A BPN' like subtilase variant comprising one or more of the following alterations:

a) deletion of residues VPG*EPST (SEQ ID NO: 41) (positions 51-58) in Savinase (BPN' numbering) and subsequent insertion of residues TVGTNFTD (SEQ ID NO: 40) (positions 56-63) from TA41 subtilase between F50 and Q59 in Savinase,

b) deletion of residues ALNNSI (SEQ ID NO: 43) (positions 74-79) in Savinase (BPN' numbering) and subsequent insertion of residues NGGTGS (SEQ ID NO: 42) (positions 83-88) from TA41 subtilase between A73 and G80 in Savinase,

c) deletion of residues ASGSGSV (SEQ ID NO: 45) (positions 98-104) in Savinase (BPN' numbering) and subsequent insertion of residues DDGSGYA (SEQ ID NO: 44) (positions 107-113) from TA41 subtilase between G97 and S105 in Savinase,

d) deletion of residues KQKNPSW (SEQ ID NO: 47) (positions 235-241) in Savinase (BPN' numbering) and subsequent insertion of residues WAQSPAA (SEQ ID NO: 46) (positions 264-270) from TA41 subtilase between V234 and S242 in Savinase.

40. An isolated nucleic acid sequence comprising a nucleic acid sequence, which encodes for the subtilase or subtilase variant defined or produced in any of the preceding claims.

41. An isolated nucleic acid sequence of claim 40, wherein the nucleic acid sequence is selected form the group consisting of:

a) a nucleic acid sequence having at least 40% homology with the nucleic acid sequence shown in SEQ ID NO: 20 or SEQ ID NO: 21, and

b) a nucleic acid sequence which hybridizes under low stringency conditions, preferably under medium stringency conditions, in particular under high stringency conditions, with

c) a complementary strand of the nucleic acid sequence shown in SEQ ID NO: 20 or SEQ ID NO: 21, or

d) a subsequence of any of a), b) or c) of at least 100 nucleotides.

42. An isolated nucleic acid sequence of claim 41, wherein the nucleic acid sequence has at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology with the nucleic acid sequence shown in SEQ ID NO: 20 or SEQ ID NO: 21.

43. An isolated nucleic acid construct comprising a nucleic acid sequence of any of claims 40-42, operably linked to one or more control sequences capable of directing the expression of the polypeptide in a suitable expression host.

44. A recombinant host cell comprising the nucleic acid construct of claim 43.

45. A method for producing the subtilase or subtilase variant of any of claims 1 to 39, the method comprising:

a) cultivating the recombinant host cell of claim 41 under conditions conducive to the production of the subtilase variant; and

b) recovering the variant.

46. A detergent composition comprising a subtilase or subtilase variant of any of claims 1 to 39 and a surfactant.

47. Use of a subtilase or subtilase variant of any of claims 1 to 39 in cleaning or washing applications.